

## **Biological Response to the Dynamic Spectral-Polarized Underwater Light Field**

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## LONG-TERM GOALS

Camouflage in marine environments requires matching all of the background optical properties: spectral, intensity and polarization components— all of which can change dynamically in space and time. Some of the most sophisticated examples of camouflage occur in our oceans, where precise regulation of spectral and polarized reflectivity is achieved through poorly understood mechanisms. Our research investigates the biological challenge of camouflage in the near-shore littoral zone and near-surface marine environments in two distinct water types found in coastal environments around the globe (oligotrophic and eutrophic). We aim to characterize the dynamic light field along with the behavioral and cellular response of camouflaging animals in these environments. Our long-term goal is to identify the biological pathways for concealment against the underwater spectral-polarized light field enabling us to identify design principles for future naval camouflage.

## OBJECTIVES

- (1) Measure and model the underwater spectral-polarized light field in oligotrophic and eutrophic systems
- (2) Quantify the biological response in fish and cephalopods to these dynamic underwater optical environments
- (3) Identify the internal controls and structural mechanisms that coordinate the camouflage response in fish and cephalopods

## APPROACH

Our first aim is to **measure and model the underwater spectral-polarized light field**. For this first objective, field measurements in two distinct field sites, Keys Marine Laboratory in Florida (oligotrophic) and Port Aransas, Texas (eutrophic), will be carried out to measure and quantify the extent of polarization and spectral features that can be expected in different water conditions, including impacts of depth, turbidity, algae, colored dissolved organic matter (CDOM), and how rapidly the light

field changes. Field measurements will be made by Dierssen (UConn), Sullivan (URI, in collaboration with Twardorski of Wetlabs, Inc.), Ahmed and Gilerson (CCNY). These measurements will be used to refine development of, and make comparisons to, theoretical expectations from a fully 3-D radiative transfer model that solves for each of the polarization elements of the Mueller matrix transformation of the Stokes vector by (Kattawar, Texas A & M), as well as to set boundary conditions for laboratory experiments.

The spectral-polarized light field will be measured by the simultaneous deployment of a comprehensive optical suite. We will deploy a hyper-spectral multi-angular Stokes vector spectroradiometer (Ahmed, Gilerson) to measure the degree of polarization as a function of the scattering angle (Figure 1). Water optical properties will be measured by Dierssen and Sullivan in parallel using ac-s for hyper-spectral water absorption and attenuation and LISST for particle size distributions. Finally, the volume scattering function (VSF) will be measured by Sullivan and Twardowski using the MASCOT (Figure 2).

The first modeling objective of this proposal is to calculate the complete Mueller matrix/Stokes vector for any set of oceanic and atmospheric conditions for any region of the ocean. We will then use this modeling approach (i) to predict the 3-D light field; (ii) to calculate experimental conditions to measure biological responses; and (iii) to investigate the nature of the light field as it interacts with cells within the skin. Our approach is to modify the 1D Monte Carlo codes of Kattawar (1) and the RayXP code of Zege (2) to develop a 3-D Monte Carlo model with full Mueller matrix treatment. Our modeling efforts will advance this effort to the full 3-D realm where confinement geometry and boundaries can now be modeled, both spatially and temporally.

Our second objective is to **quantify the biological response to these dynamic optical environments** by field observations and laboratory measurements of vertebrate and invertebrate animals from our two field sites. For this second objective, we will quantify background matching of animals in their native near-shore and near-surface environments using a diver-operated video polarimeter (Cummings), and also in a laboratory setting that allows us to recreate and manipulate optical measurements recorded in the field (Cummings, Dierssen, Sullivan, Ahmed, Gilerson, Seibel, Kattawar). From these laboratory measurements we will quantify the precision by which live animals alter their spectral and polarized reflectance to match rapid changes in light or substrate properties.

We will quantify the spectral and polarized response of our biological focal species to changes in the optical field both in the wild (to identify the range of response and candidate species) and in the lab (to precisely quantify the response). We will (a) first make observations in the field using a field-imaging-polarimeter (dual angled high resolution video camera with polarimeter heads) in an underwater housing; (b) bring organisms into the field laboratories for initial quantification of spectral-polarization response on live, awake animals; and (c) we will conduct a comprehensive quantification of the spectral-polarized dynamic response to changes in the optical field. For (c), with the aid of polarizing and spectral filters we will reconstruct the underwater light fields of the different optical habitats in large experimental chambers and measure the animal's response to temporal changes in the spectral-polarized components using a hyperspectral imaging (Dierssen in collaboration with G. Johnsen, Fig 3) and high-speed video camera.

Our third objective is to **identify the internal control and structural mechanisms that coordinate the camouflage response**. Here we plan to provide novel advances in our understanding of camouflage control by (a) examining both iridophore and chromatophore control processes for specific

background radiance matching (spectrum and polarization plane) in live, awake animals during the adaptation process (Cummings), (b) thoroughly examining local or peripheral control features (Seibel, URI; Gilly, Stanford), and (c) developing a novel 3D Monte Carlo model to describe how the spectral-polarized light field interacts with cells within the skin (Kattawar).

Using both field-studied and model organisms, we will employ two approaches: (i) A calcium-imaging optical technique to track the neural control pathway in coordinating a camouflage response in awake animals as well as prepared tissue (Seibel; Gilly; Cummings), (ii) Green-Flourescent Protein (GFP)-labeling of specific cell types (iridophores, melanophore and xanthophore) in zebrafish mutant lines to image the real-time response of these cells orchestrating a camouflage response (Cummings). Through these approaches we will characterize the internal control features regulating camouflage in both fish and cephalopods. We will also develop a novel use of Mueller matrix modeling to calculate the interaction of the light field within animal tissues (Kattawar).

## WORK COMPLETED

- a) We have convened for an initial planning meeting in August 2009 at UT. This was a joint meeting between the two MURI awards for BAA 08-019 Topic #7 (N000140911053, N000140911054). All Co-PIs were in attendance (Dierssen, Seibel, Kattawar, Gilly, Sullivan, Ahmed, Gilerson, and collaborator Twardowski) to discuss scientific collaborations and logistical arrangements.
- b) We have initiated development of the Underwater Video Polarimeter (Cummings).
- c) We have initiated model development of the fast radiative transfer code as outlined by Zege et al. In addition, we have begun the development of a 3D Monte Carlo code capable of modeling the local light field within tanks and the open ocean (Kattawar).
- d) We have deployed an *in situ* squid camera to capture live cephalopod signaling and camouflage responses by an oceanic squid in its natural environments from the surface to at least 100 m depth using only natural light. This work was carried out in collaboration with the National Geographic Crittercam group (Gilly). It is the first time that natural chromatophore behavior of the Humboldt squid at depth has been observed without the use of artificial lighting, human divers or submersible vehicles.
- e) We have identified candidate camouflage fish species from our eutrophic field site (Port Aransas, TX, Cummings) and have initiated spectral-polarized lab measurements.
- f) We made polarization measurements in coastal waters (Gilerson, Ahmed).
- g) We have identified vendors and began initial planning for outfitting the imager for laboratory experiments and radiometer with polarization filters. We are working to determine the light requirements for use of the imager in laboratory and field settings and preliminary results are positive as to the signal:noise capabilities of the sensor (Dierssen).

## RESULTS

- (i) We have done some numerical simulations to reconstruct the sky radiance distributions. In our simulations, we assumed a solar zenith angle of 60 degrees, a conservative Rayleigh atmosphere with

an optical depth of 0.25, a flat ocean surface, and an ocean with a single scattering albedo of 0.5, an optical depth of 10. The light scattering in the ocean is governed by the Heyney-Greenstein scattering phase function with an asymmetry factor of  $g = 0.95$ . The simulated radiance  $I$ , Stokes parameters  $Q$ ,  $U$ , and degree of linear polarization  $P$ , as well as field measurements, are shown in Fig. 4.

(ii) We have done some preliminary simulations using the 3D Monte Carlo code and showed that an aquarium creates certain aspects in the light field which are not found in a natural environment, and hence this must be taken into account in further studies. The 3D Monte Carlo code utilizes the radiative transfer reciprocity relation to calculate the photon path from the detector to the source. Figures 5 & 6 show a 2 by 2 by 2 meter tank with various objects within; with water properties are similar to the Sargasso Sea. Light reflection off the aquarium glass is a large factor in the light field within the tank.

(iii) Polarization measurements of light in coastal waters were made using the hyper-spectral multi-angular Stokes vector spectroradiometer shown in Fig. 1 with the goal of acquiring statistics of polarization characteristics for variable coastal environments which can then be used as model inputs as well as to reproduce specific water conditions in the tanks. Simultaneously water optical properties were measured by WET Labs package which includes ac-s, bb-9 and CTD instruments and LISST instrument for particle size distribution. Experiments were conducted in the Hudson River, NY in July 2009. The example of degree of polarization as a function of scattering angle and for 3 different azimuth angles at 1 m below the water surface are shown in Figure 7.

iv) Large Humboldt squid ( $> 80$  cm mantle length) were outfitted with a girdle-package carrying a low-light b/w video camera as well as depth and temperature sensors plus 3-dimensional accelerometers (all sampled at 1 Hz, Figure 8). Videos revealed squid flicking display that is visually similar to the shallow underwater light-field (Figure 9).

## **IMPACT/APPLICATIONS**

Understanding the underlying controls of biological camouflage may be used in the future to simulate the camouflage response artificially to enhance clandestine naval operations underwater. Such naval operations may include anti-submarine warfare, special operations, clandestine reconnaissance, and harbor security operations.

## **RELATED PROJECTS**

The CCNY group also studies polarization characteristics of light in water through another award from ONR N00014-08-1-0325 for years 2007-2010. These studies are mostly related to the development of remote sensing techniques with polarization sensitive instruments and represent one of the multiple tasks and directions pursued under this award.

The CCNY efforts will be also leveraged by other studies which are part of the NOAA Interdisciplinary Scientific Environmental Technology Cooperative Science Center (ISET) funded by NOAA through the Education Partnership Program to Minority Serving Institutions. These studies are mostly related to the development and tests of new sensors and technologies for remote sensing and other applications.

Our collaborator (M. Twardowski, WET Labs) is involved in a number of related projects including investigating the dynamics of subsurface volume scattering functions and the underlying particle composition via inversion methods (ONR); investigating the dynamics of scattering by subsurface bubble populations and other particles in the S. Ocean (NASA); developing improved remote sensing water quality algorithms for coastal waters (NASA); developing compact, low power sensing tools for ocean observing platforms (ONR); developing a microscopic holographic camera for optically relevant particles (NOPP); developing a surfzone optical drifter measuring attenuation and scattering (ONR); and developing optical prediction models for the surfzone (CEROS).

Dierssen is collaborating with the Jet Propulsion Laboratory in a NASA-funded project to build a coastal imaging spectrometer within the next two years that would have applications to this research, at no cost to the project.

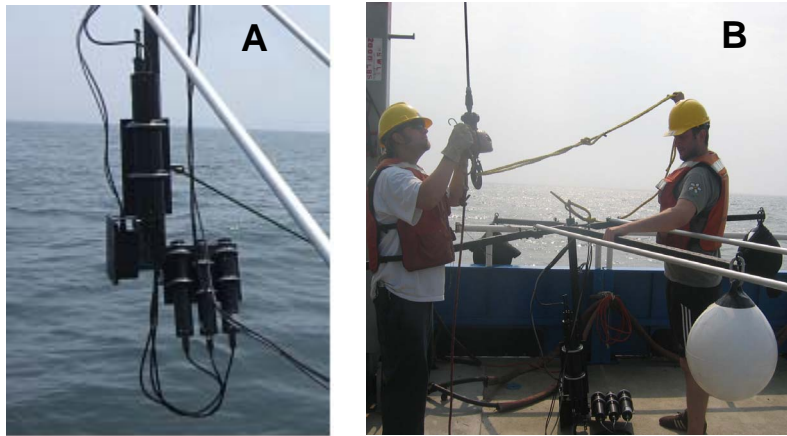
Seibel and co-PI, Sönke Johnsen are involved in an NSF-funded project to study optical physiology of transparent zooplankton in relation to oxygen, CO<sub>2</sub> and temperature. The methodological developments will be complimentary to the present project.

Gilly will continue collaborative efforts with National Geographic Crittercam to study *in situ* chromatophore displays that may be related to camouflage and communication in Humboldt squid. Discussions are underway with Greg Marshall, Vice President, and Kyler Abernathy, Director of Research, to continue this work as a regular research project. Preliminary data discussed in this report were obtained as part of a NGTV filming of an episode of a popular show, 'Dangerous Encounters with Brady Barr' to be aired on the NG cable channel. Although these efforts were not described in the original proposal, the ability to obtain natural behavioral data from this oceanic squid will significantly enhance this project.

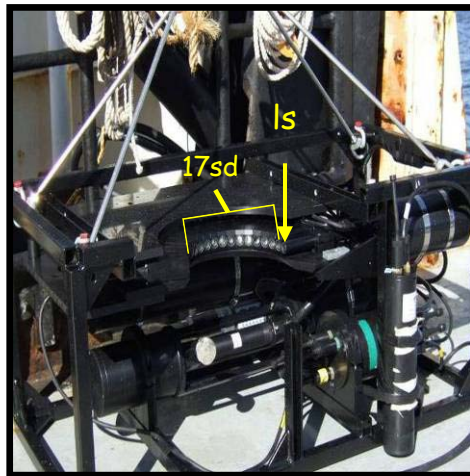
Gilly and Seibel (and Kelly Benoit-Bird, Oregon State University) are collaborators on a NSF-funded project that is focused on the physiological ecology of the oceanic Humboldt squid in regard to hypoxia tolerance. Laboratory and field methods include neurophysiology (Gilly), biochemistry (Seibel) and active acoustics (Benoit-Bird). Development of a field laboratory to carry out some of these experiments (and ones proposed for this project) at the Instituto Tecnológico Superior de Mulege in Santa Rosalía, Baja California Sur, Mexico is being carried out. Construction costs for this project are pending with NSF.

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**Figure 1. (A) Stokes vector spectroradiometer with 3 radiance sensors and data logger. (B) Instrument with horizontal arms, buoys and polls for the azimuth angle control.**



**Figure 2. MASCOT**  
**[ The image shows the volume scattering function measuring device MASCOT and highlights the 17 independent scattering detectors (sd) and lasor source (ls)]**

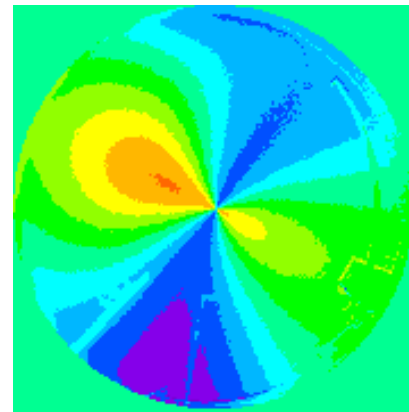
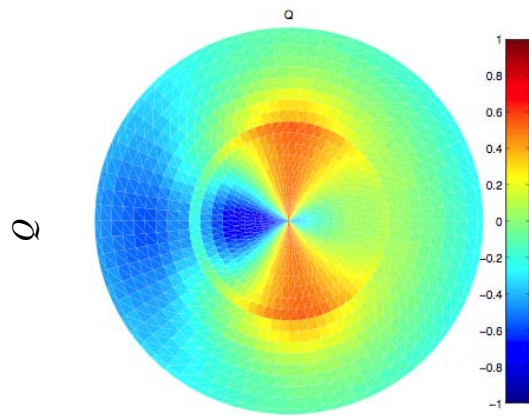
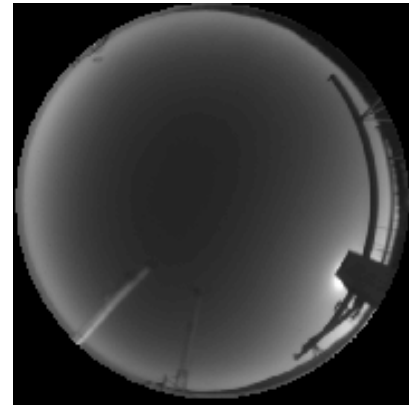
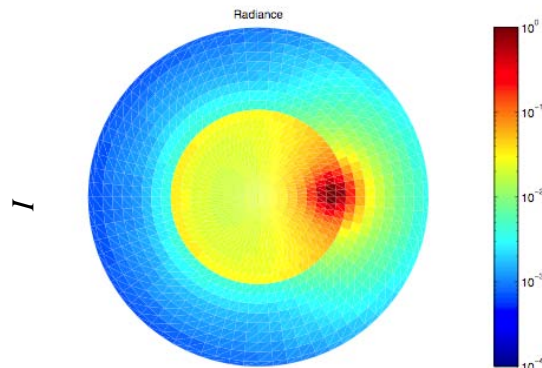


**Figure 3. Hyper-spectral imager**

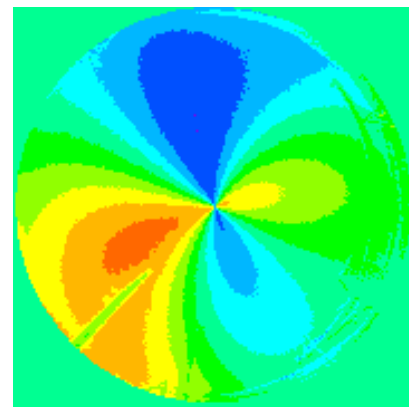
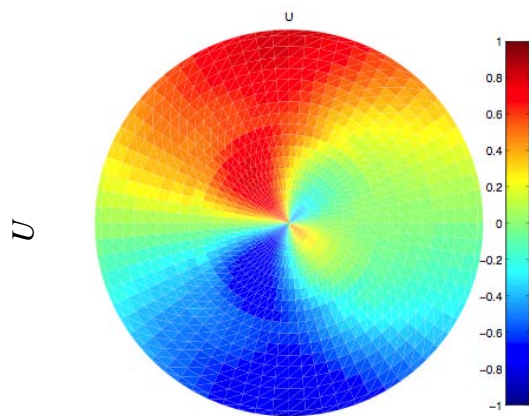


## Numerical Simulations

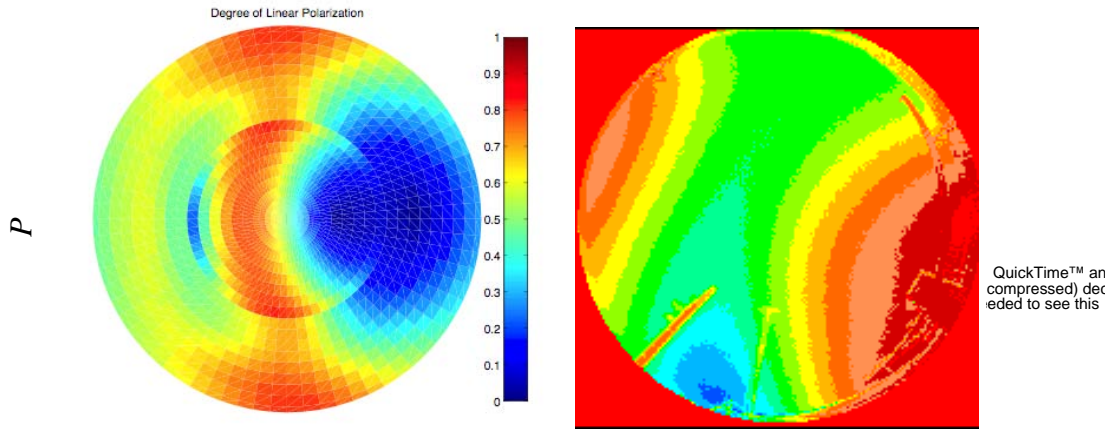
## Measurements



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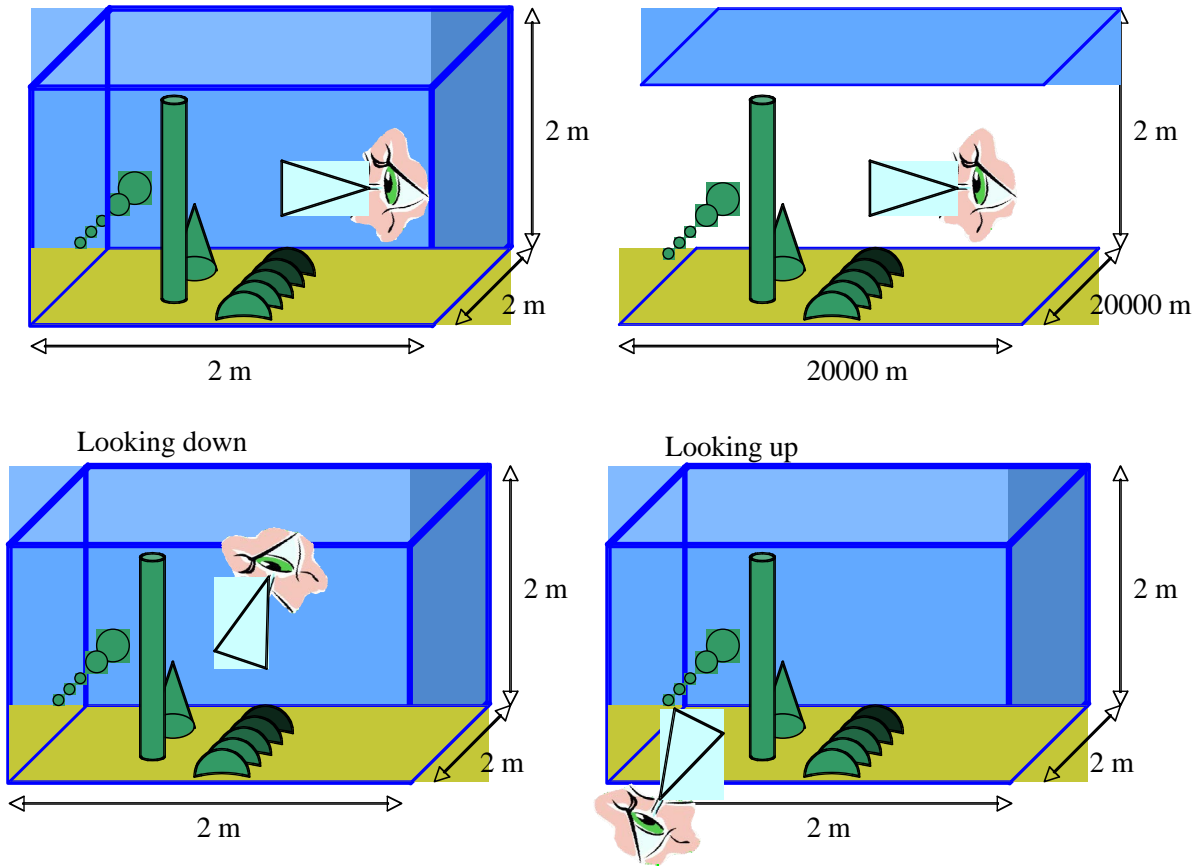


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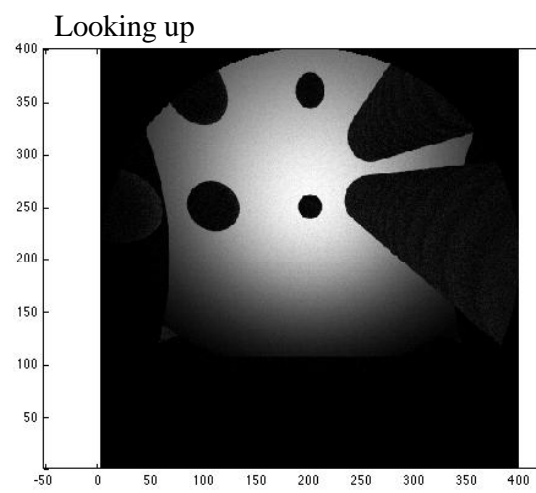
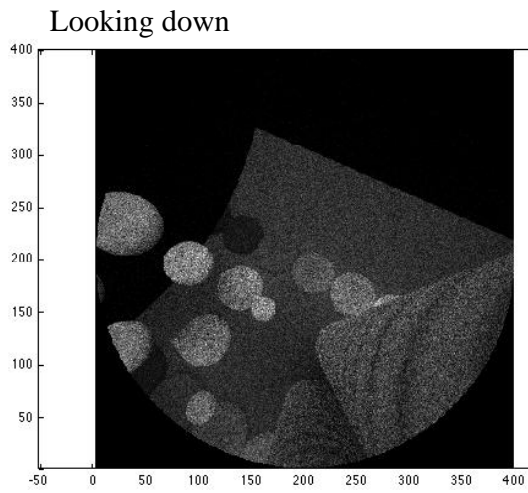
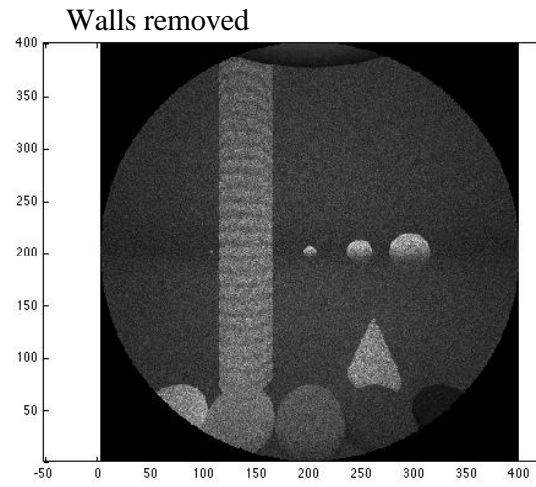
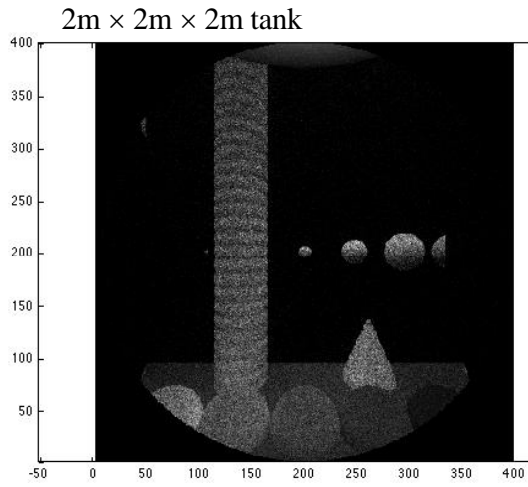


**Fig. 4. The angular distributions of the downward radiance ( $I$ ), Stokes parameters  $Q$  and  $U$ , and the degree of linear polarization ( $P$ ), as given by a Monte Carlo simulation (left panel) and by measurements (right panel).**

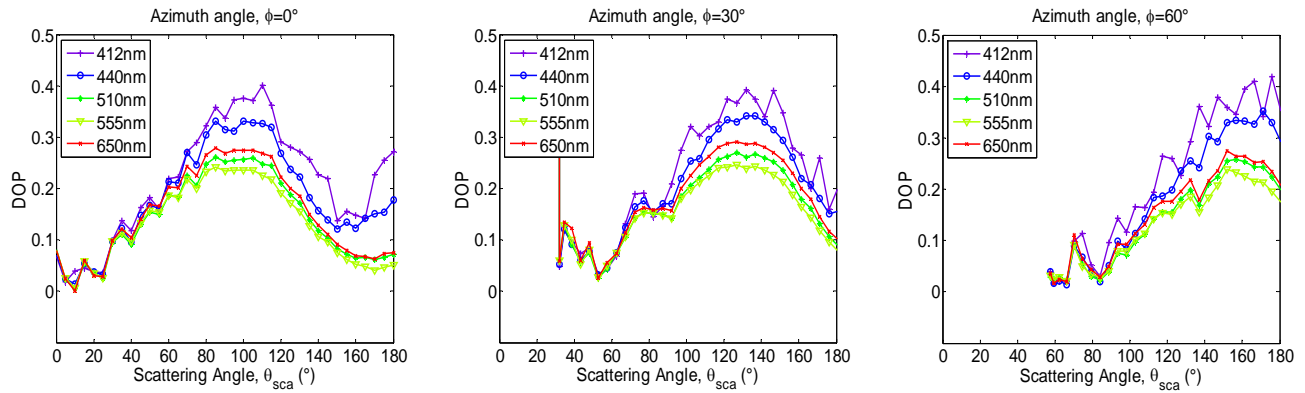
*[Figure 4 shows comparisons of the angular distributions of  $I$ ,  $Q$ ,  $U$ , and  $P$  given by Monte Carlo simulations compared to field measurements. It is obvious from Fig. 4 that the two sets of distributions have a good deal of similarity if the patterns given by measurement are rotated counterclockwise by an angle of 30 degrees. These comparisons imply that our Monte Carlo code is quite accurate in predicting the distribution of the polarized light field in a coupled atmosphere-ocean system. Moreover, one can notice that in the simulated results, there is a sharp circular boundary in each pattern. This is the boundary of the Snell's window, which is the conical region where the radiance directly transmitted through the surface could reach. In the outer region, on the other hand, there is only multiple-scattered radiance, which is substantially lower than the directly transmitted radiance. In the results obtained in the field measurements, however, this boundary disappears as the existence of the ocean waves blurs the images.]*



**Fig. 5. Schematic of a 2 by 2 by 2 m tank with spheres, cylinder, cone, and hemispheres. Each panel corresponds to a different viewing direction.**

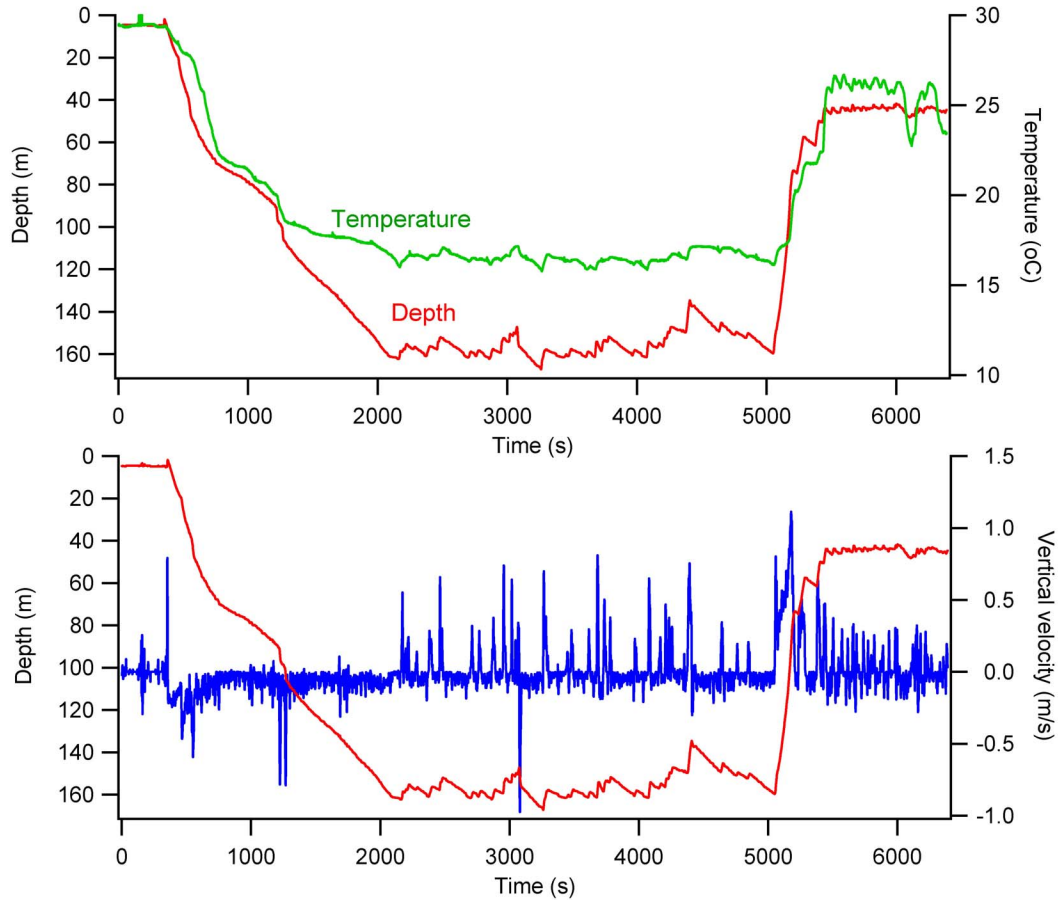


**Fig. 6.** The corresponding simulated images of the schematics shown in Fig. 5, with each panel showing a different viewing angle. Reflections from the glass walls are a prominent feature in the simulations.



**Figure 7. Degree of polarization as a function of scattering angle for various wavelengths and azimuth angles (Hudson River, NY July 2009).**

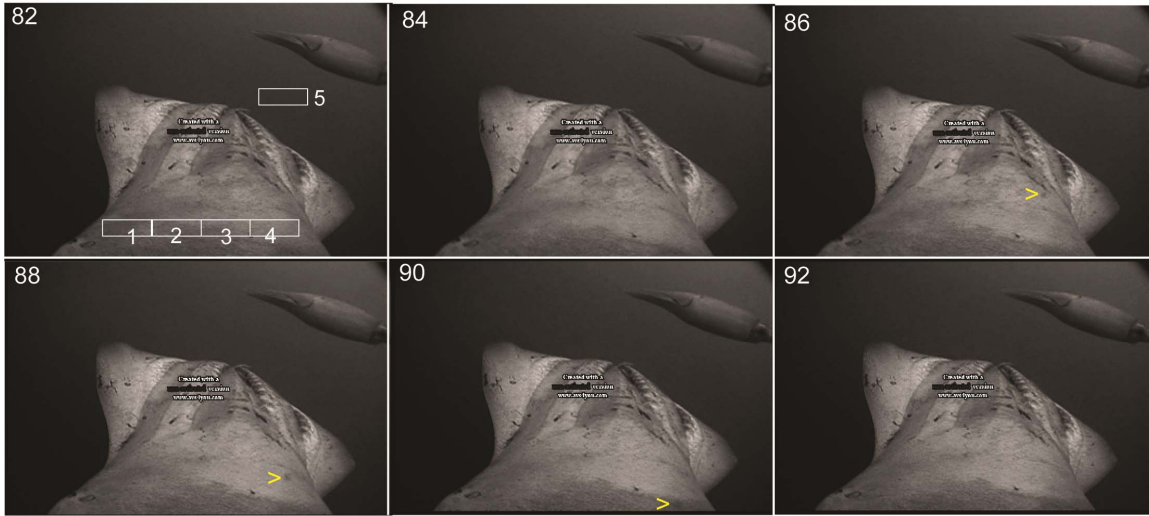
**[ For the data collected for Figure 7, this station chlorophyll concentration was  $< 2 \text{ mg/m}^3$ , mineral concentrations was about  $\text{mg/l}$ . It is shown that maximum of the degree of polarization is about 40% in the blue part of the spectrum with smaller values at other wavelengths. The data are being processed together with the data of optical properties and particle size distribution.]**



**Figure 8. Depth and temperature records from a free-swimming Humboldt squid carrying a Crittercam pack. Lower panel shows velocity spurts characteristic of squid locomotion.**

*Sampling at 1 Hz.*

*[Figure 8 shows the depth and temperature profiles collected from the crittercam package. The package detaches under timed control and floats to the surface where it is located with directional VHF radio gear. 3 successful deployments were made during 5-7 September 2009 in the Gulf of California (Santa Rosalia, Baja California Sur). The camera package was constructed by the National Geographic Crittercam group. Two deployments were made during daylight hours with no artificial lighting. Each deployment lasted ~ 2 hours; the complete depth/temperature record for Deployment 3 (daylight) is shown in Fig. 8. The lower panel also displays vertical velocity, (computed as the derivative of the depth trace). The pulsatile velocity spurts interspersed with periods of essentially passive sinking/gliding, and the ‘depth-maintenance’ behavior between 2000-5000 s, are quite comparable to the pattern of swimming we have seen in many recordings from pop-up archival tags (Ref. 3 and in preparation). This suggests that the squid was not significantly impaired by the camera pack.]*



**Figure 9. Images from a video clip during flickering behavior in a Humboldt squid at 40 m depth (right-most portion of Fig. 8).**

*[ Figure 9 shows elements of the two main types of chromatophore activity that were routinely seen. The most basic type of activity corresponds to a ‘flickering’ pattern of an irregular nature that has a rough temporal coherence in overall shade across the body, but individual elements appear and disappear in a seemingly random fashion. This flickering is difficult to demonstrate in a series of still images, but it is visually obvious in the real-time video clip. Figure 9 shows an example (every other frame) from the squid in Deployment 3. The arrow heads in panels 86 -90 (frame numbers) indicate small spots of active chromatophores that appeared in these frames and dissappeared 1-2 frames later. Elements such as these impart a pseudo-chaotic nature to the underlying flickering.*

*From this preliminary analysis it appears that the flickering is not strictly phase-locked between different patches of skin, and the overall rhythm is thus somewhat irregular in both space and time. This flickering display is visually similar to the shallow underwater light-field due to interaction of sunlight with a roughened water surface, a phenomenon of considerable importance to the studies proposed here. Footage from these experiments begins at the surface, where both variations in the light-field and chromatophore flickering are evident. As the squid descends, variations in light-field are dampened out. Further analysis will compare properties of flickering at different depths, and improved ways of analyzing the spatial and temporal aspects of this phenomenon will be developed.]*